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Factors controlling quantitative supercritical fluid extraction of environmental samples

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ABSTRACT

The development of quantitative supercritical fluid extraction (SFE) methods for the recovery of organic pollutants from environmental samples requires three steps: quantitative partitioning of the analytes from the sample into the extraction fluid, quantitative removal from the extraction vessel, and quantitative collection of the extracted analytes. While spike recovery studies are an excellent method to develop the final two steps, they are often not valid for determining extraction efficiencies from complex real-world samples such as soils and sediments, exhaust particulates, and sludges. SFE conditions that yield quantitative recoveries of spiked analytes may recover $<10\%$ of the same analytes from real-world samples, because spiked pollutants are not exposed to the same active sites as the native pollutants. Because of the heterogeneous nature of environmental samples, the partitioning step may be controlled by analyte solubility in the extraction fluid, kinetic limitations, and/or the ability of the extraction fluid to interrupt matrix–analyte interactions. While the interactions that control SFE rates from heterogenous environmental samples are not well understood, a generalized scheme for developing quantitative SFE methods is proposed based on interactive considerations of the collection efficiencies after SFE, fluid flow parameters in the extraction cell, analyte solubility, extraction kinetics, and analyte–matrix–extraction fluid interactions. The proposed development scheme includes increasing SFE extraction rates by the use of more polar fluids than CO_2 such as $CHClF_2$, the addition of organic modifiers to CO_2, and the use of high temperature extractions with pure CO_2. Validation of quantitative extractions based on multiple extraction methods (SFE followed by liquid solvent extractions) is also described.

INTRODUCTION

The interest in using supercritical fluids as a replacement for conventional liquid solvents for the extraction of organic pollutants from environmental samples has increased rapidly because of the need to reduce liquid solvent wastes as well as to perform more rapid sample preparations [1,2]. Acceptance of supercritical fluid extraction (SFE) by the regulatory community has begun as demonstrated by the recent approval of the first SFE method as a replacement for conventional Soxhlet extraction of total petroleum hydrocarbons (TPH) by the US Environmental Protection Agency [3].

While an increasing number of quantitative applications of SFE for the extraction of environmental pollutants has been reported in recent years, reported recoveries are often low and there has been little consistency in the SFE conditions (*e.g.*, fluid choice, presence and identity of modifiers, pressure, temperature, extraction flow-rate) among the various reports. Review of the available literature demonstrates that SFE conditions that successfully extract a specific pollutant from one environmental sample may not yield quantitative recovery from a different matrix. (Similar inconsistencies exist for conventional liquid solvent extraction methods, but are rarely investigated and discussed.) For example, extraction with pure CO_2 has yielded quantitative recovery of polychlorinated biphenyls (PCBs) from polyurethane foam (PUF) sorbent resins [4], while the use of similar SFE conditions only resulted in *ca.* 60% recoveries of the same PCB congeners from

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sediment [5]. It is becoming increasingly clear that high solubility of a particular species in the supercritical fluid is not a sufficient condition to yield high extraction efficiencies, and that the ability of the supercritical fluid to overcome matrix–analyte interactions is often more important than high solubility for achieving quantitative recoveries. While the interactions of pollutant molecules with a sorbent resin may be expected to be relatively homogeneous, pollutants may interact with several different binding sites (each having different binding strengths) with heterogeneous environmental solids. Even when pollutant molecules are efficiently extracted, volatile and semi-volatile organics can easily be lost during the collection step since the analytes are generally collected from the depressurized fluid at a high gas flow-rate.

The present paper describes factors that we have found to be important in developing quantitative SFE methods for extracting common pollutants from environmental matrices. The discussion will focus on the extraction of heterogeneous real-world samples (such as soil, sediments, sludges, and air particulates), since the potential analyte–matrix interactions are more complex than those expected for sorbent resins. Attempts are made to explain the differences in the extraction behavior of analytes from different matrices, and a general approach to developing quantitative SFE methods for complex environmental solids is presented. While the understanding of supercritical fluid–analyte–matrix interactions is far from complete, it is hoped that the present work will provide some useful guiding principals for the development of quantitative SFE methods for complex environmental samples.

EXPERIMENTAL

Except for the spike recovery studies, all samples contained native (not spiked) pollutants. Certified reference samples including urban air particulate matter (SRM 1649) and river sediment (SRM 1939) were used as received from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Contaminated soils and a petroleum waste sludge were used as received except that the soils were sieved through a 3.35-mm (6 mesh) screen to remove rocks and sticks before extraction.

All SFE extractions were performed using either ISCO Model 260D or 100D syringe pumps connected to either an ISCO Model SFX2-10 extraction unit or extraction cells from Keystone Scientific (Bellefonte, PA, USA) which were placed into a tube heater or a chromatographic oven to control temperature. Flow-rates through the extraction cells were controlled at the desired values of ca. 0.1–3 ml/min (measured as liquid flow at the pump) by 10–15 cm lengths of fused-silica tubing having inner diameters of 10–50 μm. Unless otherwise noted, all extracts were collected in 3–5 ml of a suitable organic solvent [acetone for GC–electron-capture detection (ECD) determinations of PCBs, perchloroethylene for infrared determinations, and methylene chloride for GC–MS and GC– flame ionization detection (FID) determinations].

Extracts were analyzed using Hewlett-Packard Model 5890 GCs equipped with FID and ECD systems. GC–MS analyses were performed with a Hewlett-Packard Model 5988 GC–MS system. Chromatographs were equipped with HP-5 (25 m × 320 μm I.D., 0.17 μm film thickness) or J&W DB-5 (60 m × 250 μm I.D., 0.25 μm film thickness) columns. TPH concentrations in extracts were determined using a Foxboro Model MIRAN-1A infrared spectrometer (Foxboro, MA, USA) as described earlier [6].

The relative extraction rates of spiked *versus* native polycyclic aromatic hydrocarbons (PAHs) were performed by spiking 1–10 μl of a methylene chloride solution containing [$^2H_8$]naphthalene, [$^2H_{10}$]phenanthrene, [$^2H_{10}$]pyrene, [$^2H_{12}$]chrysene, and [$^2H_{12}$]benzo[*b*]fluoranthene onto the sample of interest (each of which contained native PAHs), and either extracting after 10 min or after a 14-h waiting period. The relative extraction rates were determined by collecting timed fractions, adding 1-chloroanthracene as an internal standard, and determining the concentration of each deuterated PAH spike and each native PAH by monitoring their molecular ion by GC–MS.

RESULTS AND DISCUSSION

Conceptual steps in an SFE

The quantitative extraction of a particular pollutant from an environmental solid can be viewed as a three step process: First, the analyte must be efficiently (and rapidly) partitioned from the sample

matrix into the bulk supercritical fluid. Second, the analyte must be swept from the sample extraction cell. Finally, the analyte must be efficiently collected in a form that is compatible with the method used for analysis of the extract. While the first step is controlled by the chemistry of the system, steps two and three are essentially controlled by the mechanics or "plumbing" of the extractor including (but not limited to) the dimensions of the extraction cell (and sample size), the flow-rate of the supercritical fluid, and the efficiency of the collection device. Because the physiochemical processes that control SFE extraction (and collection) efficiencies are not well understood, particularly for heterogeneous environmental samples, and because of the large variety of SFE extraction and collection methods that are used, a discussion which includes all the relevant variables is beyond the scope of this paper (and indeed, is not possible with the present understanding of SFE processes). Therefore, the following discussion will attempt to present guidelines which we have found to be useful in evaluating and developing the important steps in SFE.

Trapping efficiencies. Since the first two steps in the SFE experiment listed above can not be accurately evaluated until the third step is quantitatively efficient, the collection of extracted analytes will be addressed first. Although trapping of analytes using SFE has been performed by "on-line" techniques including SFE–GC, SFE–supercritical fluid chromatograph (SFC), and SFE–LC [1,2,7–9], the vast majority of applications have utilized "off-line" trapping either in a cryogenic or sorbent trap, or (most commonly) by trapping in a small volume of liquid solvent [1]. Each collection method (with the possible exceptions of SFE–SFC and SFE–LC) depends on depressurizing the compressed supercritical fluid to ambient conditions with a coincident deposition of the analytes in (or on) the collection media. Since, for example, a 1-ml/min flow of supercritical CO_2 depressurizes to *ca.* 500 ml/min of gaseous CO_2, the collection step essentially becomes a problem in efficiently trapping the analytes from a high-flow gas stream. Although the analytes (and depressurized fluid) are cooled because of the expansion upon depressurization, trapping efficiencies can be very low depending upon the method used for collecting the extracted analytes.

While the loss of volatile analytes seems most likely, even relatively non-volatile species can be lost during the collection step. Each collection method has potential loss mechanisms associated with the phenomena used for trapping. For example, off-line trapping mechanisms have generally poor recoveries of very volatile analytes (*e.g.*, hexane, benzene), but the losses of volatiles with cryogenic trapping can be particularly severe. With certain designs of cryogenic traps, losses of >95% of compounds as non-volatile as chrysene (b.p. 440 °C) have been reported [9]. Sorbent traps must quantitatively retain all of the analytes of interest as well as allow them to be quantitatively recovered after collection. Quantitative retention on sorbent traps is particularly difficult when organically-modified CO_2 is used for the extraction, because the organic modifier (*e.g.*, methanol) becomes a liquid solvent upon depressurization and can itself elute the target analytes from the sorbent resin during the SFE step, resulting in low apparent recoveries [10]. The collection efficiencies (particularly of more volatile components) obtained using the most common collection method, liquid solvent trapping, can be affected by the identity of the collection solvent, solvent volume and temperature, collection vial design, the flow-rate of the extraction fluid, and the use of restrictor heaters (to reduce restrictor plugging, see refs. 11–13). Unfortunately, *all* trapping methods used for SFE can suffer from less-than-quantitative collection efficiencies which are often (and unfortunately) attributed to poor extraction rather than collection efficiencies. Because of the potential for poor collection of analytes after SFE (regardless of the collection method used), the first major step in developing any SFE method should be the testing (and if necessary) the development of quantitatively efficient collection methods. Appropriately designed spike recovery studies will not only aid in developing an efficient collection system, but will also determine if the target analytes have sufficient solubility to extract under the SFE conditions selected initially. However, as discussed below, they are not an appropriate method for developing quantitative extraction conditions because of large potential differences in the matrix–analyte interactions experienced by spiked and native molecules.

The extraction conditions to be used for the real-world samples should be used to extract the ana-

lytes of interest spiked at known concentrations onto a relatively inert matrix (*i.e.*, the spiked matrix should retain the spiked analytes until the SFE extraction is begun, but should not retain the spiked analytes during the SFE extraction since the goal is to evaluate only the collection method). For example, to evaluate the liquid solvent collection conditions used for the representative pollutants shown in Table I, *ca.* 18 μg of each species was spiked onto sand, extracted with the conditions that were expected to be used for real-world samples, and collected with the various test solvents [11]. Since the method was to be used for wet soil samples, the restrictors and solvent were mildly heated with a heat gun to avoid restrictor plugging expected from ice formation. As shown in Table I, recoveries of the analytes in hexane and methanol were particularly poor, and could have been mistakenly evaluated as poor extraction (rather than collection) efficiencies. Also note that, in addition to the identity of the collection solvent, additional collection conditions (including extraction fluid flow-rate, heating methods used to avoid restrictor plugging, collection solvent volume and temperature, and collection vial shape) may affect the collection efficiencies [11-13]. For example, heating the collection solvent and restrictor with a heat gun limited the collection efficiencies in the best solvent (methylene chloride) to *ca.* 75–90%. However when ice formation was avoided by keeping the collection solvent temperature from cooling below 5°C using a heating block (rather than with a heat gun), the collection efficiency increased to >98% for all of the test analytes using only 3 ml of methylene chloride as the collection solvent (Table I).

Losses that occur with collection in a liquid solvent may occur both because a particular analyte molecule is never trapped in the solvent, or because trapped molecules are purged from the collection solvent by the high gas flow of the depressurized extraction fluid. The purging losses of analytes from the collection solvent can easily be tested by preparing a suitable standard solution in the collection solvent and purging the solution with the extraction fluid in the same manner as that used for sample extractions. However, a previous study has demonstrated that the majority of losses can be attributed to inefficient partitioning of the analytes from the depressurized extraction fluid rather than to the purging of trapped analytes from the collection solvent [12], demonstrating that the best test of a collection system is the spike recovery study described above, rather than the purging study.

Each different commercially-available (and laboratory-built) collection system can have greatly different collection efficiencies, and the extraction conditions themselves (*e.g.*, flow-rate, type of restrictor used, extraction fluid identity, extraction temperature, and the presence and identity of an organic modifier) can affect the collection efficiencies. Because of the large number of experimental variables that can affect collection efficiencies, the

TABLE I

EFFECT OF SOLVENT ON COLLECTION EFFICIENCY OF REPRESENTATIVE SEMIVOLATILE POLLUTANTS

Collections were done with mild heating of the restrictor to avoid plugging from ice formation. The methylene chloride samples were also collected by placing the collection solvent in a 5°C temperature block rather than heating to avoid ice formation. Results are adapted from ref. 11.

Analyte	SFE collection efficiency			
	Hexane	Methanol	CH_2Cl_2	CH_2Cl_2 (5°C)
Phenol	43 ± 2	55 ± 12	77 ± 2	98 ± 1
1,2-Dichlorobenzene	46 ± 5	58 ± 13	78 ± 5	100 ± 2
2-Nitrophenol	57 ± 5	61 ± 8	80 ± 5	99 ± 1
Pyrene	80 ± 5	58 ± 7	90 ± 3	99 ± 5
Benzo[*ghi*]perylene	71 ± 2	67 ± 11	93 ± 2	99 ± 4

determination of the quantitative abilities of the collection device must be determined using appropriate spike recovery studies prior to further development of the SFE method. Fortunately, even with relatively simple (and inexpensive) collection methods using a few ml of liquid solvent, quantitative collection (>90%) of analytes as volatile as monoterpenes, n-octane, naphthalene, and phenol are relatively simple to achieve [11–13]. When more volatile analytes are of interest, the use of sorbent trapping or on-line SFE–GC techniques should be investigated [1,2,7,8].

Effect of extraction flow rate and cell design. Once quantitative collection conditions have been developed for the analytes of interest, the effects of the "step 2" (sweeping the analytes out of the cell) experimental parameters can be evaluated. Factors that could potentially control the rate at which an extracted analyte is swept through the sample cell include the volume of the cell (and associated dead volume not occupied by the sample), cell orientation, and flow-rate of the supercritical fluid.

First, the extraction cell should be selected to minimize the dead volume of the system, since in general, this will allow larger samples to be extracted with lower extraction fluid flow-rates (thereby reducing the amount of fluid needed and simplifying the collection of more volatile analytes). However, the exact cell size needed for various samples is often not available. In such cases, filling the cell with an inert material (*e.g.*, clean sand) at the extraction fluid inlet end to reduce the void volume of the cell may be useful. Alternatively, simple considerations of flow patterns during SFE indicate that an extracted analyte should experience the least dead volume possible to minimize the time required for its removal from the cell. Therefore, if insufficient sample is available to fill the extraction cell, the dead volume experienced by the analyte will be minimized by holding the extraction cell in a vertical position, and flowing the extraction fluid from the top to the bottom.

The effect of cell (and extraction fluid flow) orientation on the removal of an easily extracted alkane (*n*-tridecane) from a 2-g sample of sand placed in a 10-ml extraction cell is shown in Fig. 1. All extractions were performed in duplicate with a flow-rate of supercritical CO_2 of 1.5 ml/min (measured at the pump). Note that when the cell was placed vertically and the fluid was pumped from the top to the bottom, essentially all (>95%) of the tridecane is recovered after only *ca.* 3 ml (2 min into the extraction) of fluid has passed through the cell (vertical "down-flow" in Fig. 1). Since the void volume above the sample was *ca.* 8.5 ml, this rapid recovery demonstrates that the linear velocity of the fluid was sufficient to prevent significant mixing of the extracted tridecane with the CO_2 present in the cell above the sample. In contrast, when the extraction was performed with the fluid flow from bottom to top, the recovery of the tridecane is retarded by *ca.* 10 min, presumably because the extracted tridecane mixes in the *ca.* 8.5 ml of fluid present above the sample (vertical "up-flow" in Fig. 1). Similarly, the poorer flow patterns that exist when the cell is placed in the horizontal position also result in much slower recovery of the tridecane than when a vertical "down-flow" configuration is used (Fig. 1). While the dead volume considerations shown in Fig. 1 may seem obvious and trivial, it is interesting to note that the majority of commercial SFE instruments available to date utilize either vertical "up-flow" or horizontal cell orientations rather than the vertical "down-flow" orientation shown to be superior for partially-filled cells.

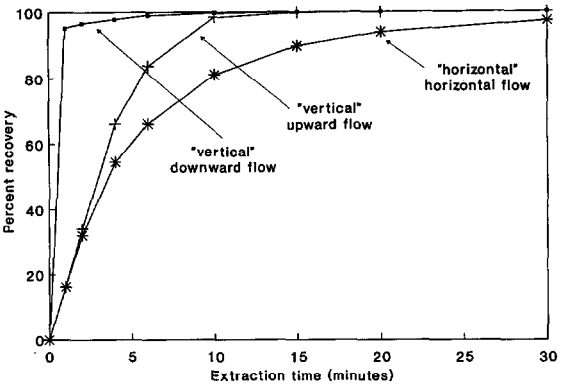

Fig. 1. Effect of cell orientation and extraction fluid flow direction on the extraction rate of *n*-tridecane from a 2-g sample of sand placed in a 10-ml extraction cell.

The example shown in Fig. 1 demonstrates the potential range of effects of cell orientation, flow direction, and cell dead volume on the recovery of an analyte that is very rapidly partitioned from the matrix into the extraction fluid under the SFE con-

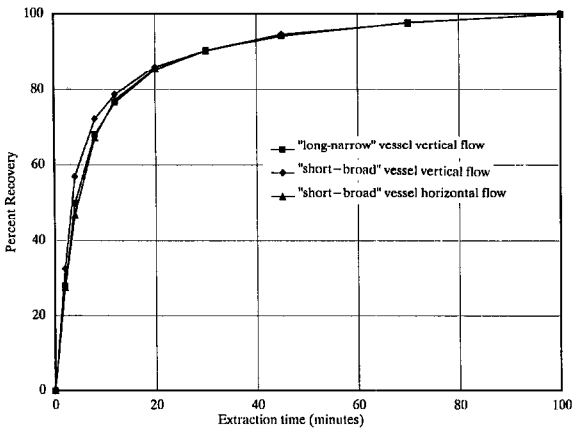

Fig. 2. Effect of cell orientation and cell dimensions on the extraction rate of fluoranthene from a 3-g sample of railroad bed soil. The "long-narrow" (132 mm × 5 mm I.D.) and "short-broad" (33 mm × 10 mm I.D.) vessels each had a volume of 2.5 ml. Extraction efficiency (100%) was based on the final amount extracted after 100 min.

ditions used. However, when extraction cells are completely filled with the sample, the effect of cell orientation (and shape) is reduced as shown in Fig. 2 by the extraction of fluoranthene from railroad bed soil using a "short-broad" and a "long-narrow" extraction cell that have identical volumes [11]. While small differences in extraction rates from sorbent resins using different cell dimensions have been reported [14], our experience with more than one hundred non-homogeneous environmental samples (*e.g.*, sludges, soils and sediments, exhaust and air particulates) have nearly always shown a kinetic limitation in the partitioning step (discussed in more detail below) that far outweighs any small effects that might result from extraction cells having different dimensions (but the same volumes). Thus, the vast majority of real-world samples that we have investigated show little, if any, detectable effect on the extraction rate of the native analytes based on cell shape or orientation when sample cells are kept full (Fig. 2).

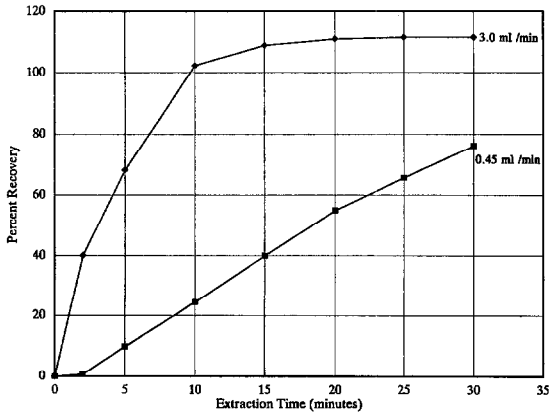

Fig. 3. Effect of SFE flow-rate on the extraction of used motor oil from a 5-g sample of soil. Extractions were performed with 340 atm CO_2 at 80°C as per the TPH method recently approved by the US Environmental Protection Agency [3,6]. Extraction efficiency (100%) was based on 4 h of Soxhlet extraction using trichlorotrifluoroethane.

The effect of fluid flow-rate on SFE extraction rates can either be nearly negligible, or can be very important depending on the process that controls the overall rate of extraction from a particular real-world sample. Assuming that the cell orientation and dead volume considerations are properly addressed as discussed above, two limiting cases for the effect of the fluid flow-rate can be imagined. First, the extraction flow-rate may be directly related to the rate at which analytes are recovered from the sample, or second, the fluid flow-rate will have no significant effect on the SFE extraction rate (as long as the fluid flow is sufficient to transport extracted analytes out of the cell). Fig. 3 shows the effect of supercritical CO_2 flow-rate on the extraction rate of a sample of the first type, *i.e.*, spilled motor oil hydrocarbons from soil (as determined by infrared spectrometry [6]). As shown in Fig. 3, the rate at which the hydrocarbons are extracted is closely related to the flow-rate of the supercritical CO_2, with higher extraction flow-rates (3.0 ml/min *vs.* 0.45 ml/min) yielding faster recoveries. In contrast, the extraction of PAHs from a railroad bed soil and PCBs from a river sediment show virtually no dependence on the flow-rate of supercritical CO_2 as shown in Fig. 4. Little if any differences in the extraction rate was observed for flow-rates of 0.3 to 0.9 ml/min for fluoranthene from the soil (Fig. 4, top). When the flow-rate was dropped to *ca.* 0.15 ml/min, the recovery rate was slower, but since the void volume of this sample was *ca.* 1.5 ml, the slower apparent recovery rate was simply a result of inefficient sweeping of the cell. However, when the flow-rate was sufficient to sweep the dead volume of the cell every few min (*i.e.*, 0.3 ml/min or greater), further increases in the extraction fluid flow-rate

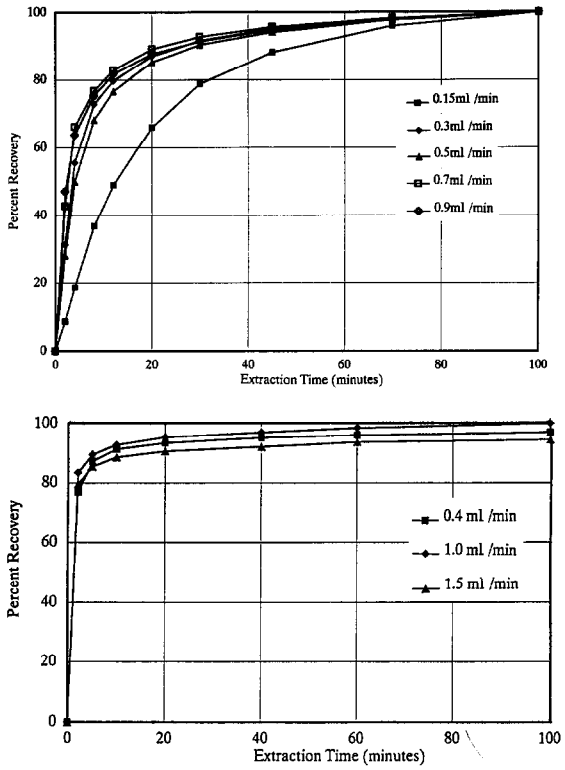

Fig. 4. Effect of SFE flow-rate on the extraction rate of fluoranthene from a 3-g sample of railroad bed soil (top) and of 2,3′,4,4′,5-pentachlorobiphenyl from river sediment (bottom). The soil sample was extracted with 400 atm CO_2 at 50°C. Extraction efficiency (100%) was based on the final amount extracted after 100 min. The recovery of the 2,3′,4,4′,5-pentachlorobiphenyl was based on the highest recovery achieved using 100 min of extraction with CO_2 at 200°C, which was slightly higher (113%) than the recovery of the same species reported by NIST based on two sequential 16-h Soxhlet extractions [20].

yielded no increase in the extraction rate. Similarly, no significant difference was observed in the extraction rate of 2,3′,4,4′,5-pentachlorobiphenyl from the sediment when the flow-rates were 0.4–1.5 ml/min (Fig. 4, bottom).

The results shown in Figs. 3 and 4 clearly demonstrate that different mechanisms control the extraction rates of these two samples. Although the mechanisms that control SFE recoveries of analytes from heterogeneous environmental samples are not nearly well enough understood to fully explain the different behavior of these two samples, our experience with a wide variety of samples indicates that some useful generalizations can be made. Samples that show a high degree of dependence on the extraction fluid flow-rate (as shown in Fig. 3) generally have very high concentrations of analytes which appear to limit the extraction rate because of solubility limitations in the extraction fluid. For example, the motor oil-contaminated soil had a total hydrocarbon concentration of *ca.* 80 mg/g (determined by both SFE and conventional Soxhlet extraction for 4 h using trichlorotrifluoroethane). Since the concentration of these analytes was very high, it is likely that the bulk of the hydrocarbons were not exposed to active sites on the soil matrix and were therefore more "available" for extraction. Therefore, the extraction problem was primarily one of solvating the hydrocarbons, and the extraction rate was increased by exposing the sample to larger volumes of the supercritical CO_2 extraction solvent per unit time.

In contrast, the railroad bed soil sample shown in Fig. 4 contained a total PAH concentration of *ca.* 100 μg/g (major species ranged from phenanthrene to PAHs with molecular masses of 252), so the majority of the individual PAH molecules could interact with active sites on the soil matrix. Since the flow-rate of the extraction fluid had virtually no effect on the extraction rates of the PAHs (as long as the sample void volume was swept every few min), the extraction rate appears to be limited by the kinetics of the partitioning process between the soil matrix and the extraction fluid rather than being limited by the ability of the CO_2 to solvate the PAHs. In our experience, this type of behavior (*i.e.*, extraction rates that are relatively independent of fluid flow-rate) generally applies to heterogeneous environmental samples as long as the concentration of the pollutants is not so high that interaction of the majority of individual pollutant molecules with the matrix active sites is prevented simply by having too high of pollutant concentrations on the sample.

In a practical sense, the extraction rate behavior exhibited by a particular sample at different flow-rates is useful to determine minimum flow-rates that will yield efficient recoveries. Flow rate studies are also useful to determine the reasonable upper sample size that can be extracted (*e.g.*, larger samples have larger associated void volumes in interstitial spaces, and therefore require higher flow-rates simply to sweep the sample void volume every few

min). Extractions that are controlled primarily by the partitioning kinetics (rather than having large amounts of available extraction fluid for the solvation step) can potentially be efficiently extracted in the static mode (that is, with no continual flow of the extraction fluid) provided that a short dynamic extraction is performed after the static extraction step simply to flush the extracted analytes out of the extraction cell. When extraction rates are controlled mostly by the fluid flow-rate, the use of extraction cell volumes (or any measurement of the total volume of fluid passed through the sample) is a useful parameter to describe an SFE method. However, for the majority of the heterogeneous environmental samples we have investigated, the extraction rate does not depend significantly on the fluid flow-rate, and the total volume of extraction fluid passed through a sample has little relevance to extraction efficiency since the contact time of the sample and the fluid is more important than the amount of extraction fluid that is used.

Finally, the results of the flow-rate studies provide insight as to whether the efficient extraction of a particular sample depends more on the partition constant between the fluid and the matrix active sites (*i.e.*, the thermodynamics of the solvation process), or on increasing the rate at which partitioning occurs between the matrix and the extraction fluid. Such information is useful in developing quantitative SFE extraction conditions as discussed below.

Analyte partitioning from matrix into extraction fluid. The least understood step that controls the SFE efficiencies obtained from heterogeneous environmental solids is the partitioning of the pollutant molecules from the active sites in the sample matrix into the supercritical fluid (step 1). Because of the large number of possible interactions that might occur between the pollutant molecules and an environmental matrix, a fundamental understanding of these partitioning processes has been impossible to attain. However, a preliminary (although admittedly naive) description of the processes that control SFE rates can be useful in developing quantitative SFE methods for complex environmental samples. The consideration of three general factors; analyte solubility, kinetic limitations, and analyte–matrix–extraction fluid interactions is useful for attempting to understand the extraction process in support of the development of quantitative SFE conditions.

The first (and most obvious) requirement of an SFE condition is the ability of the extraction fluid to solvate the target analytes. While suitable solubility data in supercritical fluids is not available for the majority of environmentally-interesting analytes, a recent review contains a sufficient number of related solubilities to allow some generalizations to be made [15]. First, organic pollutants that are sufficiently polar and non-volatile that they cannot be analyzed with conventional capillary GC generally do not have sufficient solubility to be efficiently extracted with pure CO_2 under conventional SFE conditions [*e.g.*, 300–600 atm (1 atm = 101 325 Pa), 45 to 80°C]. (A notable exception to this general rule is fat components such as triglycerides.) For example, the use of pure CO_2 to extract ionic species such as the surfactant linear alkylbenzenesulfonate (LAS) shows little if any recoveries because of their low solubility. However, as their solubility in the supercritical CO_2 is increased by the addition of an organic modifier or ion pairing reagents, high extraction efficiencies can be obtained [16,17].

Just as polar and high-molecular-mass analytes generally do not have sufficient solubility to dissolve in pure CO_2, species that are amenable to GC analysis generally do have sufficient solubility to make their extraction using pure CO_2 seem likely. For example, the solubilities of several common pollutants are shown in Table II. Based on the solubility data, it should be possible to quantitatively

TABLE II

ESTIMATED SOLUBILITIES OF REPRESENTATIVE ORGANICS IN SUPERCRITICAL CO_2 AT 400 atm AND 50°C

Solubilities were estimated based on the tabulations given in ref. 15.

Species	Solubility (mg/ml)
Docosane	320
Phenol	170
p-Chlorophenol	140
Hexachloroethane	230
Diphenylamine	31
Naphthalene	160
Phenanthrene	13
Pyrene	3
Dibenzothiophene	11

extract a 1-g sample contaminated with 13 000 μg/g (*ca.* 1%, w/w) of phenanthrene using only 1 ml of supercritical CO_2 at 400 atm and 50°C. Since the relevant environmental concentrations of such pollutants are typically much lower (*e.g.*, ng/g to μg/g), one might expect that pure CO_2 extractions of such species would be highly efficient. While many of the early SFE investigations were based on the assumption that attaining high solubility in the supercritical fluid should be sufficient to obtain high extraction efficiencies from environmental samples, the unfortunate truth is that high solubility in the extraction fluid is generally not a sufficient condition to yield high extraction efficiencies [1,5,9,18–21], and such results clearly demonstrate that additional factors for real-world samples must be considered.

In addition to the obvious need for adequate solubility, a successful extraction condition must overcome the interactions between the analyte and the matrix to affect a favorable partitioning into the supercritical fluid (loosely termed the "thermodynamic problem" for this discussion). The extraction condition must also cause this partitioning to occur rapidly (on the time scale of the extraction experiment) for high recoveries to occur in a reasonable time (loosely termed the "kinetic problem"). Obviously, these factors depend on the nature of the interactions between the analytes and matrix components. Unfortunately, the nature of analyte–matrix interactions between pollutants and heterogeneous environmental samples is not well understood, and the potential for different types of interactions seems nearly endless as composition of individual matrix components is considered. For example, the pollutants in a soil sample may be associated with a variety of inorganic (*e.g.*, alumina, silica) and/or organic (*e.g.*, humic and fulvic) active sites, each with different binding strengths. (In contrast, the number of possible interactions between analytes and sorbent resins is relatively limited.) In addition, the extraction of the pollutants may be inhibited by physical barriers including being located in interstitial micropores in the soil particles (or between clay plates), being covered by (or associated with) bulk organic material, or by being coated with droplets of water that may need to first be extracted before the analyte is accessible for extraction. The differences in potential interactions multiply when matrices in addition to soil are considered (*e.g.*, sludges and exhaust particulates), however, in every case both the "thermodynamic" and "kinetic" problems need to be solved to develop a successful extraction method.

The effect of extraction flow-rate on the extraction rate can be used to investigate whether the major limitation to achieving rapid extractions is primarily a "thermodynamic" (*i.e.*, the distribution of the analyte between the supercritical fluid and the sample matrix at equilibrium) or "kinetic" (i.e, the time required to approach that equilibrium) problem. For samples that show a dramatic increase in extraction rate when the extraction fluid flow-rate is increased (Fig. 3), the kinetics of the extraction process appear to be fast, and the extraction will be improved by increasing the proportion (partition constant) of the analytes in the extraction fluid or by simply exposing the sample to more fluid. In contrast, if there is no large effect of fluid flow-rate on the extraction rates, it appears that the kinetics of the desorption process is slow, and these slow kinetics limit the overall extraction rate more than the equilibrium distribution of the analyte between the matrix and the extraction fluid [18,19,21–23]. Finally, it must be remembered that many different interactions of a pollutant species with a sample matrix are possible, and the lack of understanding of the processes that control analyte–matrix interactions and therefore control SFE extraction mechanisms makes *a priori* prediction of quantitative extraction conditions impossible. However, sufficient work has been reported to allow a somewhat logical approach to developing an SFE method to be suggested as discussed below.

Validating a quantitative SFE method

In addition to the large number of different analyte–matrix combinations and interactions, the development of any extraction method for real-world environmental samples is severely limited by the fact that it is simply not possible to know the exact concentration of any target pollutant on any sample. Since samples with known concentrations of native (not spiked) pollutants are not possible to obtain, the development and validation of a quantitative extraction method for environmental samples is generally based on one of the three following approaches, each depending on assumptions that may or may not be valid:

(i) Determining the recovery of known concentrations of spiked compounds from the sample (or similar) matrix. This approach assumes that spiked analytes behave like native analytes during the extraction, and also assumes that the spike is not lost between spiking and extraction from processes such as volatilization.

(ii) Comparison of the recoveries of native analytes with those achieved using conventionally-accepted extraction methods (including the use of standard reference materials). This approach assumes that the conventional method is quantitatively efficient.

(iii) Perform multiple sequential extractions of the same sample. This approach assumes that the final extraction performed removes all of the native analytes and that no additional analytes are associated with the sample by stronger interactions than the analytes that were already extracted.

While all three approaches have been used to develop (and to attempt to validate) SFE and other extraction methods, the complexity of environmental samples and their potential interactions with pollutant molecules makes heavy reliance on any one validation technique unwise. (Obviously, the limitations of extraction validation methods apply to any extraction method, and are not associated only with SFE.) Perhaps the least reliable technique for validating the quantitative abilities of an extraction method is the use of spike recoveries [21], simply because the spiked analytes are not exposed to (*e.g.*, contaminated soils) or formed with (*e.g.*, soot samples) the same matrix active sites as are the native pollutants. The potential errors in validating an SFE method based on spike recovery studies are shown in Fig. 5 by the relative extraction rates of native naphthalene and spiked [$^2H_8$]naphthalene from three different samples: urban air particulate matter, a petroleum waste sludge, and soil from a railroad bed. Each sample was spiked with the [$^2H_8$]naphthalene at the same approximate concentration as the native naphthalene, and the samples were extracted for 30 min with pure CO_2 at 400 atm and 60°C. As shown in Fig. 5, quantitative recovery (>90%) of the spiked [$^2H_8$]naphthalene was achieved after only 5 min of extraction, while the recovery of the native naphthalene was *ca.* 55%, 18%, and 5%, for the soil, air particulate, and waste sludge samples, respectively. If a commonly-

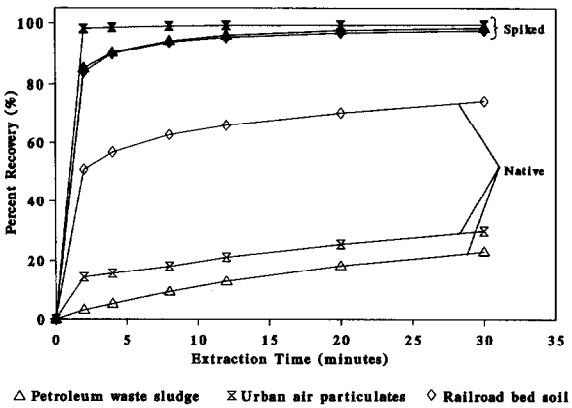

Fig. 5. Extraction rates of spike [$^2H_8$]naphthalene and native naphthalene from petroleum waste sludge, urban air particulates, and railroad bed soil. Percent recoveries were based on the total amounts extracted by sequential extractions with pure CO_2 (shown in the figure) at 400 atm and 60°C followed by CO_2–10% methanol (same conditions) and finally by 14 h of sonication in methylene chloride.

applied criterium for a successful extraction method was used (*e.g.*, that >90% of the target spike was recovered), the results shown in Fig. 5 demonstrate that the same extraction time and conditions would fail to extract between 45% and 95% of the native naphthalene from the real-world samples. Even after 30 min of extraction with pure CO_2, the recoveries of the native naphthalene were only 74 ± 8%, 30 ± 5%, and 23 ± 6%, respectively, compared to >98% for the recovery of the [$^2H_8$]naphthalene spike from all three samples. The results shown in Fig. 5 also clearly demonstrate that, as discussed above, solubility in the supercritical fluid is not a sufficient extraction condition since the solubility of naphthalene is *ca.* 160 mg/ml, while the concentration of naphthalene was only *ca.* 1 μg/g, 3 μg/g, and 100 μg/g for the air particulate, soil, and sludge samples, respectively (and since *ca.* 25 ml of supercritical CO_2 was used for each extraction). It should also be noted that aging the spiked samples for 14 h compared to extracting freshly spiked samples had no effect on the extraction rates shown in Figs. 5 and 6.

While the results shown in Fig. 5 clearly demonstrate that the use of spike recoveries is not valid for determining quantitative extraction conditions, some spiked analytes do behave in a manner that is

similar to that displayed by the native analytes. For example, the SFE rates of the higher-molecular-mass PAH, chrysene, are more similar to those of the spiked [$^2H_{12}$]chrysene when extracted from the same three samples as shown in Fig. 6, although significant differences still exist between the extraction rates of the spiked and native chrysene for the air particulate and sludge samples. Since the differences shown between spiked and native PAHs must be a result of differences in the strength of the analyte–matrix interactions experienced by the individual spiked and native PAH molecules, it would seem logical that spike recovery studies may be more relevant for highly contaminated samples with relatively few significant analyte–matrix interactions (*e.g.*, the motor oil-contaminated soil sample shown in Fig. 3). However, the results shown in Figs. 5 and 6 clearly demonstrate that spike recovery studies are best used to evaluate the collection efficiencies of an extraction method, and should not be used to determine quantitative extraction conditions.

The second suggested method for validating quantitative extraction conditions is to compare the

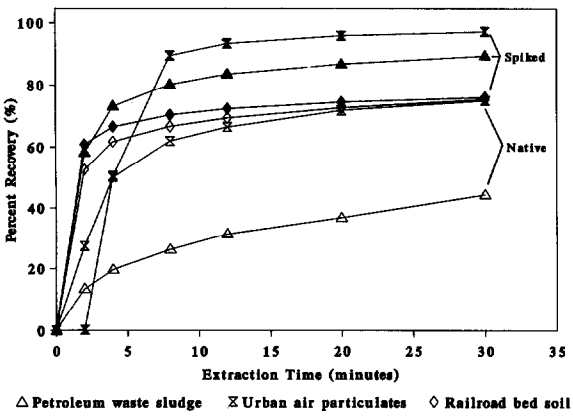

Fig. 6. Extraction rates of spike [$^2H_{12}$]chrysene and native chrysene from petroleum waste sludge, urban air particulates, and railroad bed soil. Percent recoveries were based on the total amounts extracted by sequential extractions with pure CO_2 (shown in the figure) at 400 atm and 60°C followed by CO_2–10% methanol (same conditions) and finally by 14 h of sonication in methylene chloride. The slight lag in the recovery of the [$^2H_{12}$]chrysene from the urban air particulates was a result of chromatographic retention of the spike compound on the urban air particulate sample during SFE since the spike (placed on the top of the sample) had to be eluted through the sample before recovery.

results of the proposed SFE method with those of conventional and well-accepted extraction methods such as extraction with a liquid solvent in a Soxhlet apparatus. This approach includes the use of certified reference standards, such as those available from NIST, that contain native pollutants for which concentrations have been certified based on exhaustive Soxhlet extraction and multiple analysis methods. (The use of certified reference materials to validate SFE methods for the extraction of PAHs and nitro-PAHs from urban air particulate matter, diesel exhaust particulates, and marine sediment, and PCBs from river sediment have been the subject of earlier reports, see refs. 5,20,24–26.) Perhaps the greatest advantage of certified reference materials is that investigators involved in SFE methods development can have a single "benchmark" data set for which to compare results between methods and laboratories. Similarly, when reference materials are not available, comparison of SFE results with standard liquid solvent extractions (Soxhlet or sonication) provides a somewhat consistent method to evaluate SFE results between methods and laboratories. However, it should be noted that conventional liquid solvent extractions may not yield quantitative extraction of native analytes, and therefore a highly efficient SFE extraction may yield higher recoveries than Soxhlet or sonication extraction. For example, the recoveries obtained for PAHs using SFE with $CHClF_2$ as the extraction fluid (30 min) were substantially higher from a petroleum waste sludge sample than those obtained using 18 h of sonication with methylene chloride (*e.g.*, recoveries of phenanthrene and benz[*a*]anthracene by SFE were *ca.* 120% and 150%, respectively, of those obtained from the sonication extraction [5].

The final approach to validating the quantitative abilities of an extraction method, performing multiple extractions of a single sample, can be quite misleading or very useful depending on how it is performed. Early work in SFE (including work performed in this laboratory) often used a second sequential SFE extraction performed under conditions identical to the first extraction in order to estimate the overall extraction efficiency. The assumption was that if (for example) the second extract contained substantially lower concentrations of the analytes than the first extraction, then the extraction must be nearly quantitative. While this as-

sumption may be true if all of the individual molecules of a particular analyte extracted at the same rate, this assumption is clearly not valid for the majority of environmental samples we have investigated. For example, Fig. 7 shows the extraction rate (with pure CO_2 at 400 atm and 50°C) of PCB congeners 2,3',4,4'-tetrachlorobiphenyl and 2,3',4,4',5-pentachlorobiphenyl from river sediment (NIST SRM 1939). The extraction curves show initially fast extractions, followed by increasingly slow rates of extraction as has been previously described by a kinetic model based on diffusion kinetics [22,23]. (It should be noted that diffusion of the analyte in the sample matrix is not likely to be the major limiting step for the extraction of heterogenous environmental samples since grinding the samples does not generally increase the extraction rate.) However, the model provides a useful mathematical description of the extraction rates observed for these samples. If, for example, two sequential extractions of PCB-contaminated sediment were performed for 40 and 60 min, respectively, (*i.e.*, 0–40 min and 40–100 min in Fig. 7), no significant concentrations would be detected in the second extract, which could be interpreted that the first 40-min SFE extraction was quantitatively efficient. However, this is clearly not true, since the recovery of two PCB congeners was only *ca.* 50 and 70% at 40 min (based on the values certified by NIST based on Soxhlet extraction).

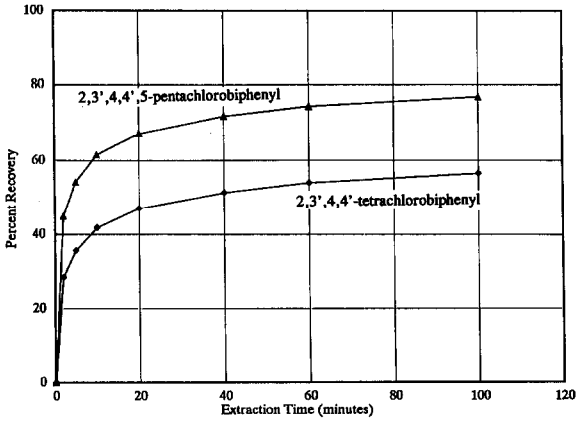

Fig. 7. Extraction rates of 2,3',4,4',5-pentachlorobiphenyl (top) and 2,3',4,4'-tetrachlorobiphenyl (bottom) from river sediment using 400 atm pure CO_2 at 50°C. Percent recoveries are based on those reported by NIST based on two sequential 16-h Soxhlet extractions.

Quantitative recovery of the PCBs from the same sample has been achieved using methanol-modified CO_2, $CHClF_2$, and high-temperature extraction with pure CO_2 [5,20]. Note that, with the exception of the high concentration motor oil-contaminated soil (Fig. 3), all of the samples discussed in this paper show similar extraction curves as those of the PCB congeners shown in Fig. 7 (*i.e.*, an initial fast extraction followed by a slow rise), which further demonstrates that the determination of quantitative recovery based on multiple sequential extractions with the same extraction condition is not valid.

In contrast, the use of multiple extractions of a single sample with a different (and presumably stronger) extraction condition can be a very useful way to validate the quantitative ability of an extraction method for real-world samples. This could include extracting the residue from an SFE extraction by Soxhlet or sonication in an appropriate liquid solvent, by extracting the residue with a "stronger" SFE condition (*e.g.*, by adding a modifier), or by a combination of the two approaches. For example, the total concentration of the PAHs in the petroleum waste sludge, urban air particulates, and the railroad bed soil discussed earlier (100% recovery for Figs. 5 and 6) were determined by sequential extraction with 30 min of pure CO_2 (400 atm, 60°C), 30 min with CO_2 modified with 10% methanol (400 atm, 60°C), and finally by 14 h of sonication of the SFE residue in methylene chloride [21]. With such an approach, if no significant concentrations of the target analytes are seen in the conventional liquid solvent extract performed on the residue, it seems reasonable to conclude that the SFE extraction was quantitatively efficient.

Developing a quantitative SFE method

The understanding of SFE mechanisms for the extraction of organic pollutants from heterogeneous environmental samples is simply not well enough developed to propose a single approach to SFE methods. However, careful consideration of the various mechanical and physicochemical aspects of SFE that are discussed above has led to a general approach that has proven very useful for the development of quantitative SFE methods in our laboratory. The following discussion attempts to list a sequential method of developing a quantitative SFE method based on the scheme shown in Fig.

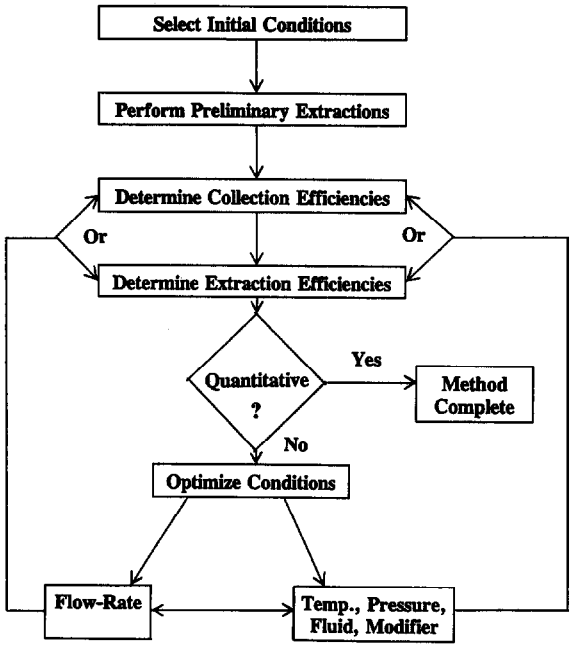

Fig. 8. Proposed interactive scheme for the development of quantitative SFE methods for complex environmental samples. Discussion of the individual steps is given in the text.

8, however, it must be recognized that such development is an interactive process and some of the steps may need to be repeated during the optimization process.

Selection of initial extraction conditions. The first methods development task is to determine the initial extraction conditions based on considerations of the polarity of the target analytes (and solubility data, if available), the matrix composition (*e.g.*, water content, organic content, mineral composition, particle size), and any literature reports of successful SFE methods for similar samples. If no relevant solubility or extraction results are available for the target analytes, initial conditions may be chosen based on the polarity of the analyte, (*e.g.*, using the general rule described above that pure CO_2 will generally solvate GC-able analytes at "normal" extraction conditions such as 400 atm and 50°C). If the analytes are fairly polar (or ionic) or have high-molecular-masses, the addition of an organic modifier (or for ionic compounds, an ion pairing reagent [16]) may be necessary to obtain sufficient solubility of the analyte in the extraction fluid. The use of a more polar fluid such as $CHClF_2$ may also be useful as discussed below, however, little data exists in the literature to help select such fluids. If no basis exists (other than the polarity of the target analytes) for selecting initial conditions, reasonable starting points (for non-polar analytes) would be extraction with 0.5 to 2 ml/min of pure CO_2 at the upper pressure limit of the SFE system (typically 340 to 650 atm, depending on the manufacturer) and a moderate temperature (50–100°C), and (for polar analytes) extraction with similar conditions with CO_2 modified with 5–10 vol.% of an organic modifier that is itself a good solvent for the target analyte, and has properties that do not interfere with the subsequent analysis (*e.g.*, the selection of modifiers with high boiling points interferes with GC analysis); if the analysis is performed by spectrometry, the modifier must not absorb at the detection wavelength. The addition of the modifier will also raise the critical temperature of the mixture, so the extractions should be performed at slightly higher temperatures (*e.g.*, 70°C for modified CO_2).

Preliminary extractions of representative samples. The choice of initial extraction conditions also should include consideration of "mechanical" problems related to the sample matrix which may occur during the extraction since the solution to these problems may also affect the collection efficiencies. For example, samples with high concentrations of water and/or extractable matrix components will likely require that linear restrictors be heated and/or that drying or dispersing agents be added to the sample to avoid plugging of the restrictor during the SFE step. Preliminary extractions of representative samples should be performed to investigate if such problems are significant. These preliminary extractions are also useful to select a "reasonable" sample size based on sensitivity requirements, sample availability (*e.g.*, 1 g is a very large sample of air particulate matter, but is a small soil sample), and sample homogeneity. (Because of the flow considerations discussed above, SFE is most easily applied to smaller samples, and samples of a few grams or less is a reasonable starting point.) It is particularly useful to perform preliminary extractions of a limited number of samples that represent the range of analyte, water, and co-extractable matrix concentrations that might be expected in future samples so that the analyst is familiar with the

types of matrix problems to be encountered. Because of the shape of the extraction rate curves generally encountered with heterogeneous environmental samples, preliminary extraction times of 30 min are useful since longer extraction times (using the same SFE conditions) generally do not yield substantially higher recoveries.

Determination of collection efficiencies. Once the preliminary extraction conditions have been determined, the collection efficiencies of target analytes which have been spiked onto a relatively non-active matrix (*e.g.*, sand) at concentrations expected to be encountered in real-world samples should be determined. Quantitative recovery of the spiked analytes will demonstrate that: (i) the collection system is efficient, (ii) sufficient flow is available to overcome the void volume effects in the cell, and (iii) the analytes are soluble enough to be dissolved in the supercritical fluid (however, this does *not* necessarily demonstrate that the fluid can extract the analytes from real samples as discussed above).

In general, the quantitative collection of analytes less volatile than *n*-octane or naphthalene is not difficult in a few ml of the proper liquid solvent [11–13], as long as care is taken in performing the collection step (although it is possible to have low collection efficiencies of non-volatile analytes when the liquid solvent collection system is poorly designed as in ref. 27). However, the collection in a few ml of solvent of more volatile compounds such as benzene and toluene is difficult (*ca.* 45% and 75% efficiency, respectively [28], and the quantitative collection of such volatile analytes may require the use of sorbent traps or on-line approaches such as SFE–GC.

If low recoveries of the spike are observed, the reason can often be determined by observing the spike recovery efficiency of a homologous series of related analytes. For example, if an extraction is being developed for PAHs, recoveries could be determined for a spike containing a range of PAHs including naphthalene (M_r 128) and representative PAHs with molecular masses of 178, 202, 228, 252, and 276. If the spike recoveries of the more volatile components are low, and the recoveries of the less volatile compounds are high, volatilization losses are indicated, and the trapping system must be improved as discussed in refs. 10–13 (losses from volatilization during the spiking step must also be considered). Conversely, if the recoveries of the volatile components are high, and the recoveries of the less volatile components are low, then the extraction condition (*e.g.*, solvation strength of the supercritical fluid) is not sufficient for the larger (less soluble) PAHs (although losses by deposition in the depressurization system may also be responsible). Regardless of the trapping method used, quantitative spike recoveries must be demonstrated before further development of the SFE method is warranted.

Determine extraction efficiency. Once high spike recoveries have been achieved, the first determination of the SFE efficiency of the target analytes from real-world samples can be performed. At this stage in the development, one hopes for good luck, *i.e.*, that the conditions that were satisfactory for spike recoveries will also yield quantitative recovery of the native analytes. Since the concentration of the native analytes can not be known in real-world samples, the definition of "quantitative" must first be determined. For reasons discussed earlier "quantitative" should not be based on spike recovery data. Therefore, the decision must be made whether to base "quantitative" recovery using the SFE method on the recovery of conventional extraction (*e.g.*, Soxhlet or sonication) results, or the use of multiple sequential extractions using different extraction techniques. If, based on the definition of "quantitative," the present SFE conditions yield satisfactory recoveries, the method can be considered complete. However, if the recoveries are not "quantitative," additional development of the method will be required (Fig. 8).

Optimizing SFE conditions. Since the spike recovery studies have already demonstrated that the target analytes are sufficiently soluble to be extracted with the initial SFE conditions, low recovery of the analytes must be based on the inability of the initial SFE conditions to efficiently overcome matrix–analyte interactions. Alternatively, the sample could contain too high concentrations of the target (and non-target) analytes that could saturate the extraction fluid. The flow-rate studies described above are a simple method to determine which of these mechanisms is predominant. If the use of higher flow-rates results in substantial increases in recovery of the native analytes, it is likely that simply exposing the sample to more fluid by increasing the extraction flow-rate (which may make analyte collection

more difficult) or increasing the extraction time will yield quantitative recoveries. Simple changes to increase the solubility of the analytes such as raising the extraction pressure (*i.e.*, fluid density) should also be considered.

However, if increasing the extraction flow-rate (or extraction time) does not yield substantial increases in the recoveries of the native target analytes (as shown in Fig. 4), the initial SFE conditions are not sufficient to efficiently overcome analyte–matrix interactions (*i.e.*, interactions not experienced by the spiked analytes). Assuming that the upper pressure of the extraction system is already being exploited, three useful parameters are available to increase the extraction rates, *i.e.*, use of different (more polar) fluids than CO_2, the addition of organic modifiers to CO_2, and increasing the extraction temperature.

Depending on the SFE system used, the effect of increasing the extraction temperature with pure CO_2 extractions can be very simple to evaluate (a simple and inexpensive approach for performing SFE at temperatures up to 200°C is described in ref. 20). Even though the solubility of the target analyte may actually decrease at higher temperatures (and constant pressure) because of lower CO_2 density, extraction at 200°C has been shown to be extremely effective in obtaining quantitative recoveries of PCBs from sediment and PAHs from air particulate matter, indicating that the kinetics of the partitioning process are improved [20]. For example, when extractions were performed for 40 min with pure CO_2 at 350 atm, increasing the extraction temperature from 50 to 200°C yielded *ca.* one-and-one-half to two-fold increases in the recovery of PCBs, and two- to six-fold increases in extraction efficiencies of the PAHs. While results of SFE with pure CO_2 at "normal" temperature (50°C) were not quantitative based on the concentrations certified by NIST (based on 32–48 h of Soxhlet extraction), extraction at 200°C yielded recoveries that generally met or even exceeded the certified concentrations [20].

Increased recoveries may also be achieved by using different SFE fluids. Unfortunately, no fluids have the attractive characteristics attributed to CO_2 (low toxicity, low reactivity, and low environmental impact), although N_2O and $CHClF_2$ may be worth investigating when recoveries with pure CO_2 are low. N_2O yields higher recoveries than CO_2 for some samples (*e.g.*, PAHs from marine sediment, chlorinated dioxins from fly ash [25,29]), however N_2O did not yield increased recoveries from some samples such as PAHs from waste sludge or PCBs from river sediment [5]. In addition, N_2O can react with easily oxidized organics, and may present a safety hazard for routine applications [30]. $CHClF_2$ (freon-22) has been the most efficient pure SFE fluid that we have encountered, and has been shown to yield excellent recoveries of nitro-PAHs, PAHs, PCBs, and even some ionic species from a variety of matrices [5,24]. Despite its excellent characteristics as an extraction fluid, $CHClF_2$ does cause fused-silica restrictors to break easily and is less desirable because of ozone destruction caused by freons (although $CHClF_2$ has a relatively low ozone-depletion potential [31]).

If higher temperature extractions or the use of alternative fluids are impractical or ineffective, the addition of organic modifiers to CO_2 is the next logical step. Although methanol has been the most often used modifier, many other potentially useful modifiers (including ion pairing reagents [16,17]) should be evaluated. Unfortunately, little information is available to aid the choice of modifiers (and their concentrations), and until the action of modifiers (and related analyte–matrix interactions) is better understood, the optimal selection of modifiers to extract complex environmental samples should be based on a survey of suitable candidates (as well as consideration of the modifier's effect on collection recoveries and on the subsequent method used to analyze the extract).

Three basic approaches to adding modifiers can be used, *i.e.* purchasing a pre-mixed cylinder of the modifier in CO_2, purchasing an SFE system (*e.g.*, dual pump) capable of modifier addition, or simply adding the modifier to the extraction cell with the sample. Unfortunately, surveying the abilities of several modifiers with the first two approaches can be quite time-consuming and expensive. While adding the modifier to the sample in the extraction cell has the disadvantage that the modifier is not continually introduced during dynamic (continual flow) SFE, its inherent simplicity and low cost suggests that the initial choice of modifier identity and concentration be based on this method. An appropriate survey of modifiers can easily and rapidly be performed using a single pump by adding an appropri-

TABLE III

ENHANCEMENT IN PAH RECOVERIES FROM URBAN AIR PARTICULATES USING DIFFERENT MODIFIERS IN CO_2

Values given are the quantities of each PAH extracted with a 5-min static extraction with the modifier listed followed by a 10-min dynamic extraction with pure CO_2 divided by the quantities of each PAH extracted from a fresh sample using a 15-min dynamic extraction with pure CO_2.

Modifier (%, v/v)	Enhancement in recovery *vs.* pure CO_2		
	Fluoranthene	Chrysene	Benzo[*ghi*]perylene
Methanol (10)	1.0	1.1	1.3
Methanol (1)	0.9	1.0	1.1
Toluene (10)	1.1	1.4	4.8
Toluene (1)	1.0	0.8	1.1
Aniline (10)	2.4	1.8	2.0
Aniline (1)	2.3	1.1	1.4

ate volume of the modifier to the extraction cell with the sample, pressurizing and performing a static extraction (no flow out the cell) for 5–30 min, then recovering the analytes with a dynamic extraction step with pure CO_2 for 10 to 30 min.

The results of such a modifier survey for the extraction of PAHs from urban air particulate matter are shown in Table III. Each extraction was performed on 400-mg samples placed in a 2.5-ml extraction cell. Each modifier was added at 1 or 10% of the cell volume, the cell was pressurized with 400 atm CO_2 (80°C), and the static extraction was performed for 5 min followed by a dynamic extraction for 10 min with pure CO_2. As shown in Table III, the enhancement of the PAH recoveries (calculated as the ratio of the individual PAHs extracted with the modifier compared to the amount extracted in 15 min with pure CO_2) with the different modifiers varied by both the polarity of the modifier, its concentration, and the individual PAH. For example, methanol was the poorest modifier for all of the PAHs, and only yielded slight improvement in recovery for benzo[*ghi*]perylene at the 10% concentration. Toluene yielded no significant enhancement for fluoranthene, however 10% toluene was by far the best modifier for the benzo[*ghi*]perylene. In contrast, 10% aniline yielded the same approximate enhancement (*ca.* two) for all of the PAHs. (It must be noted that these were only 5-min extractions in presence of the modifier so that differences between modifiers would be accentuated. A normal SFE would involve a longer contact of the modifier with the sample, and it is likely that both toluene and aniline modifiers would yield good recoveries of the PAHs.)

The addition of modifier directly to the sample may not yield as high of extraction efficiencies as dynamic extractions (using a dual pump system or pre-mixed cylinders) since the modifier is present only during the static extraction step. When the modifier acts by increasing the solubility of the analyte in the extraction fluid, a constant addition of modifier should yield higher extraction efficiencies. However, if the modifier acts primarily by facilitating the removal of the analytes from the matrix active sites (and not by increasing its solubility in the extraction fluid), the simple addition of the modifier to the sample (with a static extraction step followed by a dynamic extraction step with pure CO_2) may be sufficient and eliminate the need to purchase a dual pump system or pre-mixed fluids.

CONCLUSIONS

The development of quantitative SFE conditions is facilitated by an interactive process of developing quantitative collection conditions for the extracted analytes, determining the extraction kinetics (including effect of the extraction fluid flow-rate), and finally by determining the extraction condition that will cause efficient and rapid partitioning of the analytes from the matrix active sites into the extraction fluid. Regardless of the approach used to increase SFE recoveries, any significant changes in the extraction condition should be followed by a new spike recovery study to determine collection efficiencies (*e.g.*, higher temperature extractions may reduce the collection efficiencies of cryogenic and liquid solvent traps, the addition of an organic modifier may reduce the collection efficiency of a sorbent trap). While repeated determinations of spike recoveries may seem laborious, it has been our experience that careful attention to collection efficiencies during methods development can save great amounts of overall effort, since poor recoveries are often incorrectly attributed to the extraction process rather than the collection process (and

therefore the methods development efforts are focused on the wrong step in the SFE scheme). The speed at which the extractions can be performed (*e.g.* a typical extraction time is 30 min, and more than one extraction can be performed at a time) makes the development work faster than might be expected. Indeed, the SFE experiments can often be performed more rapidly than the analysis of the extracts (*e.g.*, a GC analysis normally requires 30 to 60 min) particularly when many fractions are collected from a single extraction as described for the extraction kinetic studies. In addition, the use of this general approach has helped our laboratory develop and validate quantitative SFE conditions for a variety of analytes ranging from volatile non-polar species to non-volatile ionic species from a large range of complex environmental matrices. In every case, SFE extraction conditions have been developed that yield high efficiencies with extraction times of 40 min or less.

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REFERENCES

1 S. B. Hawthorne, *Anal. Chem.* 62 (1990) 633A.
2 R. W. Vannoort, J.-P. Chervet, H. Lingeman, G. J. de Jong and U.A. Th. Brinkman, *J. Chromatogr.*, 505 (1990) 45.
3 B. Lesnick (US Environmental Protection Agency, Office of Solid Waste), announcement at the *EPA SFE Workgroup meeting, Washington, DC, July 17, 1992.*
4 S. B. Hawthorne, M. S. Krieger and D. J. Miller, *Anal. Chem.*, 61 (1989) 736.
5 S. B. Hawthorne, J. J. Langenfeld, D. J. Miller and M. D. Burford, *Anal. Chem.*, 64 (1992) 1614.
6 V. Lopez-Avila, J. Benedicto, N. S. Dodhiwala, R. Young and W. F. Beckert, *J. Chromatogr. Sci.*, 30 (1992) 335.
7 H. J. Cortes, L. S. Green and R. M. Campbell, *Anal. Chem.*, 63 (1991) 2719.
8 K. Jinno and M. Saito, *Anal. Sci.*, 7 (1991) 361.
9 B. W. Wright, C. W. Wright, R. W. Gale and R. D. Smith, *Anal. Chem.*, 59 (1987) 38.
10 L. J. Mulcahey and L. T. Taylor, *Anal. Chem.*, 64 (1992) 2352.
11 J. J. Langenfeld, M. D. Burford, S. B. Hawthorne and D. J. Miller, *J. Chromatogr.*, 594 (1992) 297.
12 M. D. Burford, S. B. Hawthorne, D. J. Miller and T. J. Braggins, *J. Chromatogr.*, 609 (1992) 321.
13 N. L. Porter, A. F. Rynaski, E. R. Campbell, M. Saunders, B. E. Richter, J. T. Swanson, R. B. Nielsen and B. J. Murphy, *J. Chromatogr. Sci.*, 30 (1992) 367.
14 K. G. Furton and J. Rein, *Anal. Chim. Acta*, 248 (1991) 263.
15 K. D. Bartle, A. A. Clifford, S. A. Jafar and G. F. Shilstone, *J. Phys. Chem. Ref. Data*, 20 (1991) 713.
16 J. A. Field, D. J. Miller, T. A. Field, S. B. Hawthorne and W. Gieger, *Anal. Chem.*, 64 (1992) 3161.
17 S. B. Hawthorne, D. J. Miller, D. E. Nivens and D. C. White, *Anal. Chem.*, 64 (1992) 405.
18 N. Alexandrou, J. Pawliszyn and M. Lawrence, *Anal. Chem.*, 64 (1992) 301.
19 J. Pawliszyn, *J. Chromatogr. Sci.*, 31 (1993) 31.
20 J. J. Langenfeld, S. B. Hawthorne, D. J. Miller and J. Pawliszyn, *Anal. Chem.*, 65 (1993) 338.
21 M. D. Burford, S. B. Hawthorne and D. J. Miller, *Anal. Chem.*, (1993) in press.
22 K. D. Bartle, A. A. Clifford, S. B. Hawthorne, J. J. Langenfeld, D. J. Miller and R. Robinson, *J. Supercrit. Fluids*, 3 (1990) 143.
23 K. D. Bartle, T. Boddington, A. A. Clifford and S. B. Hawthorne, *J. Supercrit. Fluids*, 5 (1992) 207.
24 T. Paschke, S. B. Hawthorne, D. J. Miller and B. Wenclawiak, *J. Chromatogr.*, 609 (1992) 333.
25 S. B. Hawthorne, D. J. Miller and J. J. Langenfeld, *J. Chromatogr. Sci.*, 28 (1990) 2.
26 M. M. Schantz and S. N. Chesler, *J. Chromatogr.*, 363 (1986) 397.
27 M. Ashraf-Khorassani, R. K. Houck and J. M. Levy, *J. Chromatogr. Sci.*, 30 (1992) 361.
28 S. E. Eckert-Tilotta, S. B. Hawthorne and D. J. Miller, *Fuel*, in press.
29 N. Alexandrou and J. Pawliszyn, *Anal. Chem.*, 61 (1989) 2770.
30 R. E. Sievers and B. Hansen, *Chem. Eng. News*, 69 (1991) 2.
31 P. S. Zurer, *Chem. Eng. News*, July 24 (1989) 7.